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i686-pc-linux-gnu May 24 2002 11:56:25

FIG. 4 demonstrates that the geometry of the agonist-induced time dependent translocation of βarr2-GFP to the plasma membrane mimicked the distribution of pre-aggregated β2ARs. This indicates that the primary site targeted by β-arrestin is the β2AR or a closely associated component.

EXAMPLE 7

Intracellular βarr2-GFP Targets Membrane Receptors

It has been postulated that phosphorylation of GPCRs by GRKs facilitates desensitization by increasing the affinity for β-arrestins. Gurevich et al., *J. Biol. Chem.* 268:16879 (1993); Gurevich et al. *J. Biol. Chem.* 268:11628–11638 (1993); Ferguson et ,al., *Can. J. Physiol. Pharmacol.* 74:1095 (1996). When expressed in HEK-293 cells and exposed to agonist, mutant Y326A-β2ARs are not significantly phosphorylated by endogenous GFKs. Barak et al., *Biochem.* 34:15407 (1995) ; Ferguson et al., *J. Biol. Chem.* 270:24782 (1995). This phosphorylation impairment in Y326A-βAR2s is reversed by overexpression of GFKs in the same cell. Menard et al., *Biochem.* 35:4155 (1996). The Y326A mutant receptor was used to investigate β-arrestin affinity in vivo; the effect of overexpressed GFK on the Y326A-B2AR interaction with βarr2-GFP was shown.

Y326A-β2AR and βarr2-GFP were co-transfected into HEK-239 cells, in the absence and presence of co-transfected GRK. If phosphorylation of GPCRs by GRKs facilitates desensitization by increasing their affinity for β-arrestins, then overexpression of GRK would result in a noticeable difference in βarr2-GFP translocation.

FIG. 5 shows the influence of overexpressed GFK on the redistribution of βarr2-GFP in HEK-293 cells expressing the Y326A phosphorylation impaired β2AR. Cells without (Row A) and with (Row B) overexpressed GRKs were exposed to agonist, and the real-time redistribution of βarr2-GFP was observed. Without added GRK, βarr2-GFP translocation in response to agonist proceeded poorly, as shown in Row A of FIG. 5. βarr2-GFP translocation in cells containing overexpressed GRK (Row B) was more robust, indicating an increased affinity of βarr2-GFP for receptor and the relationship of phosphorylation and β-arrestin activity.

EXAMPLE 8

Testing of Additional Receptors in the β2AR/rhodopsin Subfamily

Twelve different members of the β2AR/rhodopsin subfamily of GPCRs have been studied. Cells expressing a particular GPCR, and containing βarrestin-GFP chimeric proteins were exposed to known agonists for the GPCR being studied. In each case, an observable translocation of the βarrestin-GFP chimeric proteins from the cell cytosol to the cell membrane was produced within minutes following addition of the GPCR agonist (data not shown).

What is claimed is:

1. A method of detecting G protein coupled receptor (GPCR) pathway activity in a cell expressing at least one GPCR and containing β-arrestin protein conjugated to an optically detectable molecule, said method comprising detecting translocation of the detectable molecule from the cytosol of the cell to the membrane edge of the cell, wherein said translocation of the detectable molecule indicates activation of the GPCR pathway;

and wherein said detecting step comprises (i) detecting an increase in said detectable molecule at said membrane edge; (ii) detecting a decrease in said detectable molecule in said cytosol; or (iii) detecting both an increase in said detectable molecule at said membrane edge and detecting a decrease in said detectable molecule in said cytosol.

2. A method according to claim 1 wherein said detection is of an increase in the detectable signal at the membrane edge of the cell over time.

3. A method according to claim 1 wherein said detection is of a decrease in the detectable signal in the cytosol of the cell over time.

4. A method according to claim 1 wherein said translocation is detected by comparing the distribution of the detectable signal in a test cell to the distribution of the detectable signal in a control cell.

5. A method according to claim 1 wherein said detection of the detectable signal occurs over time.

6. A method according to claim 1 wherein said translocation is detected by comparing the distribution of the detectable signal in a test cell to a pre-established standard.

7. A method according to claim 1 wherein said detectable molecule is photochemically detectable.

8. A method according to claim 1 wherein said detectable molecule is biochemically detectable.

9. A method according to claim 1 wherein said detectable molecule is immunochemically detectable.

10. A method according to claim 1 wherein said detectable molecule is spectroscopically detectable.

11. A method according to claim 1 wherein said cell is a mammalian cell.

12. A method according to claim 1, wherein the cell is selected from the group consisting of bacterial cells, yeast cells, fungal cells, plant cells and animal cells.

13. A method according to claim 1 wherein the cell expresses a GPCR whose function is known.

14. A method according to claim 1 wherein the cell expresses a GPCR whose function is unknown.

15. A method according to claim 1 wherein the cell expresses an odorant GPCR.

16. A method according to claim 1, wherein the cell expresses a β-adrenergic GPCR.

17. A method according to claim 1, wherein the cell endogenously expresses a GPCR.

18. A method according to claim 1, wherein the cell has been transformed to express a GPCR not endogenously expressed by such a cell.

19. A method according to claim 1, wherein the cells are deposited on a substrate prior to detecting translocation of the detectable molecule from the cytosol to the membrane edge.

20. A method according to claim 1 wherein said cell is contained in a tissue.

21. A method according to claim 1 wherein said cell is contained in an organ.

22. A method according to claim 1 wherein the cell expresses a taste GPCR.

23. A method according to claim 1 wherein the cell is an insect cell.

24. A method according to claim 1, wherein said detecting step comprises

detecting an increase in said detectable molecule at said membrane edge.

25. A method according to claim 1, wherein said detecting step comprises

detecting a decrease in said detectable molecule in said cytosol.

26. A method according to claim 1, wherein said detecting step comprises

detecting both an increase in said detectable molecule at said membrane edge and detecting a decrease in said detectable molecule in said cytosol.

* * * * *

WEST Search History

DATE: Friday, June 14, 2002

Set Name side by side	Query	Hit Count	Set Name result set
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L5	estrogen near surface near receptor	0	L5
L4	l1 or l2 or L3	32	L4
L3	iCI 182780	28	L3
L2	Faslodex	2	L2
L1	Fulvestrant	3	L1

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=> ici 182780

L1        1200 ICI 182780

=> faslodex

L2         106 FASLODEX

=> fulvestrant

L3          46 FULVESTRANT

=> l1 or l2 or l3

L4        1280 L1 OR L2 OR L3

=> estrogen(p)surface(p)receptor

L5        1274 ESTROGEN(P) SURFACE(P) RECEPTOR

=> l4 and l5

L6          36 L4 AND L5

=> l6 and 1970-1997/py

   2 FILES SEARCHED...
L7           5 L6 AND 1970-1997/PY

=> dup rem l7

PROCESSING COMPLETED FOR L7
L8           2 DUP REM L7 (3 DUPLICATES REMOVED)

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=> ici 182,780

L9        1550 ICI 182,780
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=> l9 or l2 or l3

L10       1627 L9 OR L2 OR L3

=> l4 and l5

L11         36 L4 AND L5

=> l10 and l5

L12         48 L10 AND L5

=> l12 and 1970-1997/py

L13          6 L12 AND 1970-1997/PY

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L14           2 DUP REM L13 (4 DUPLICATES REMOVED)

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L15     326758 CELL MEMBRANE

=> l9 and l15

L16         42 L9 AND L15

=> l16 and 1970-1997/py

L17          7 L16 AND 1970-1997/PY

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PROCESSING COMPLETED FOR L17
L18           3 DUP REM L17 (4 DUPLICATES REMOVED)

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